

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: G.P. Murphy *et al.* Attorney Docket No.: NWBI135161
Application No.: 09/016737 Art Unit: 1642 / Confirmation No.: 7366
Filed: January 30, 1998 Examiner: Minh Tam B Davis
Title: ISOLATION AND/OR PRESERVATION OF DENDRITIC CELLS FOR
PROSTATE CANCER IMMUNOTHERAPY

DECLARATION OF MARNIX L. BOSCH

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TO THE COMMISSIONER FOR PATENTS:
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I, Marnix L. Bosch, state as follows:

1. I am currently the Chief Technical Officer for Northwest Biotherapeutics, Inc., the assignee of the above identified application. I was the Vice President for Vaccine Research and Development for Northwest Biotherapeutics from July 2001 until December 2006, and Vice President for Vaccine Development from 2006 until 2010. I have a Ph.D. in Medicine from the University of Leiden, the Netherlands, and an MBA from the University of Washington. In addition, I have over 40 publications in the area of virology and immunology.

2. The invention presently claimed in the above-identified application (herein "the '737 application") is directed to *inter alia*, a composition comprising an isolated cell population having human dendritic cells, wherein said cell population has been cultured in the presence of granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin 4 (IL-4), and exposed *in vitro* to a soluble prostate antigen, the cell population having an increased ability to activate T cells specific to the prostate antigen as compared to a similar isolated cell population cultured in

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the presence of granulocyte-macrophage colony stimulating factor (GM-CSF), and interleukin 4 (IL-4) that has not been exposed *in vitro* to the prostate antigen. The prostate antigen can be, for example, a lysate of LNCaP cells, a membrane preparation of LNCaP cells, a lysate of prostate tumor cells from a prostate cancer patient, a membrane preparation of prostate tumor cells from a prostate cancer patient, isolated prostate specific membrane antigen (PSMA), purified prostate specific membrane antigen (PSMA), a peptide having the amino acid sequence LLHETDSAV (SEQ ID NO: 1), a peptide having the amino acid sequence ALFDIESKV (SEQ ID NO: 2), a peptide having the amino acid sequence XL(or M)XXXXXV(or L) (SEQ ID NO: 3), where X represents any amino acid, purified prostate specific antigen (PSA), or a purified prostate mucin antigen recognized by monoclonal antibody PD41. Still further, the dendritic cells can be extended life dendritic cells, they can be cryopreserved prior to exposure *in vitro* to the prostate antigen, wherein said dendritic cells retain the ability to take up and present antigen, and the human dendritic cells can be immature dendritic cells. The dendritic cells can also be isolated from a prostate cancer patient, from a normal individual, for example, where the normal individual has been HLA matched to the patient. In addition, the T cells can be CD4⁺ or CD8⁺.

3. I understand that claims 23, 31, 32, and 33 - 37 remain rejected under 35 U.S.C. § 103(a) as being obvious over Sallusto *et al.* in view of Bigotti *et al.*, as evidenced by Inaba *et al.* In particular, the Examiner has considered the prior arguments provided by Applicants and considers them non-persuasive. Regarding the first reason for rejection, the Examiner has objected to the lack of "objective evidence" showing that Langerhans cells stained with anti-S-100 antibody are immature dendritic cells. The Examiner has noted that Bigotti *et al.* identified some S-100-staining cells directly in contact with prostate tumor glands, and most adjacent to the prostate tumor glands, citing to page 76, first paragraph and the last paragraph of page 79 bridging to page 80. In addition, the Examiner has noted that i) in the presence of antigen, immature antigen presenting cells are capable of pick up and processing the antigen citing Sallusto *et al.*, ii) it is well known in the art that necrotic cancer cells shed cancer antigens into their vicinity and circulation and iii) that Applicants do not have objective evidence that

Langerhans cells at the vicinity of prostate cancer glands did not pick up the antigen during their journey from the skin or epidermis to the vicinity of prostate cancer glands.

4. I have reviewed Bigotti *et al.* and in particular the portions of the reference cited by the Examiner. In addition, I have reviewed and am familiar with the teachings of Sallusto *et al.* I agree that the portions of Bigotti *et al.* cited by the Examiner that cells identified as Langerhans cells labeled in anti-S-100 treated sections were characterized as elongated cells sometimes with dendritic processes displaying a nuclear as well as cytoplasmic staining and that the cells were usually present as isolated elements at the periphery of the tumor or within the fibrous septa in-between the neoplastic glands. The authors also noted that anti-S-100 stained strongly the nerve trunks in the prostate. In addition, the authors noted that they found that low-grade carcinomas were very rich in HLA class II-positive, interstitial, oval to elongated cells, which were sometimes in close contact with tumor glands. These cells were identified by the authors as "mostly representing macrophages and in only small percentages LCs, as comparing the adjacent S-100-stained section". They also found that in patients classified as having prostate tumors in the intermediate group as having less abundant interstitial cells and no clear cut correlation with the immunoreactivity pattern for S-100 and HLA class II. High grade carcinomas were found to have a scarce number of HLA class II-positive cells. Sallusto *et al.* does teach that in the presence of antigen, immature dendritic cells are capable of picking up and processing antigen when contacted *in vitro*. It is also well known in the art that necrotic cancer cells shed antigens into their vicinity and into the circulation. The antigens are all antigens, including small molecules, proteins, carbohydrates, lipids, and the like that were present in the dying cell.

5. With respect to the teachings of Sallusto *et al.*, the data and conclusions were based on *in vitro* studies with monocyte-derived immature dendritic cells, whereas Bigotti *et al.* refers to an *in vivo* situation with what might be Langerhans cells of unknown status. In regard to the statement about necrotic cancer cells, it should be noted that the tumors where the Langerhans cells were identified by Bigotti *et al.* were low grade tumors and typically do not comprise necrotic cells. As such, this statement is irrelevant to any argument regarding whether any

antigen were available for pick up, processing and presentation by an antigen presenting cell of any type.

6. Bigotti *et al.* does not provide any evidence to support the induction of an anti-tumor immune response. First, Langerhans cells are known to be present in the interstitial space of most glandular tissues. Second, S-100 is not an exclusive marker for Langerhans cells, and is a marker for cells derived from the neural crest. The identification of the S-100-stained cells as Langerhans cells is questionable in Bigotti *et al.* because there is no mention of the presence of Birbeck granules. Third, Langerhans cells in any tissue stain strongly positive for HLA class II antigens, but this association is not found by Bigotti *et al.*, who state instead "we found that low-grade carcinomas were very rich in HLA-class II-positive, interstitial, oval to elongated cells, which sometimes were in close contact with tumor glands". These cells were further characterized by Bigotti *et al.* as representing macrophages and in only small percentages Langerhans cells. Fourth, there is no evidence of infiltrating T cells in the tumor, and therefore no objective evidence of the anti-tumor response postulated by the authors. As such, at most the findings of Bigotti *et al.* support a low level non-specific inflammatory environment mediated by infiltrating macrophages and as evidenced by class II expression on the tumor cells and the interstitial cells. No objective evidence is presented that supports a functional role for Langerhans cells in these tissues, or for the induction of an immune response, let alone an immune response against cancer antigens. In fact, there is no mention of cancer antigens in Bigotti *et al.*

7. It is my opinion that the overall theme of Bigotti *et al.* is that S-100-positive cells and class II-positive cells are most abundant in low grade prostate tumor, the authors merely speculate about immune mechanisms wherein Langerhans cells may act as antigen presenting cells in a neoplastic environment. Bigotti *et al.* further speculate that HLA class II molecules expressed by neoplastic glandular epithelium may interact primarily or with the aid of Langerhans cells with macrophage and secondarily with T helper lymphocytes to cause the expansion of cytotoxic T cell clones and enhancement of the antibody response to membrane bound tumor-associated antigens. The skilled artisan at the time of the present invention would

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have known that i) antigen presenting cells, including Langerhans cells, were present in normal glandular tissue, ii) when contacted by a foreign antigen the antigen presenting cell would uptake the antigen becoming activated to mature, iii) the activated Langerhans cell typically would migrate to a lymphatic tissue where the cell would complete maturation, iv) upon maturation the antigen presenting cell would present antigen to T lymphocytes, including CD4+ and CD8+ T lymphocytes, wherein the T lymphocyte is activated, and v) the activated T lymphocyte, for example a tumor infiltrating lymphocyte, migrates back to the tumor. B lymphocytes can also be activated by this process to produce antibody specific for tumor associated antigens. It is important to note that the antigen must be "foreign" and that mechanisms are present *in vivo* to prevent a response to self-antigens. There is no reasonable expectation for the skilled artisan to reasonably combine the *in vivo* observations of Bigotti *et al.* with the teaching of Sallusto *et al.* to obtain the presently claimed compositions of the '737 application.

8. It is my opinion that based on the teachings of Bigotti *et al.* and that there is no evidence that i) the S-100 stained cells are activated Langerhans cells that have picked up any antigen; ii) that the S-100 stained cells in the vicinity of the prostate tumor glands did not pick up the antigen during their journey from the skin or epidermis to the vicinity of prostate cancer glands. Further, there is no reason provided in Bigotti *et al.* for the skilled artisan to reasonably expect the S-100 stained cells of Bigotti *et al.* to have pick up and processed antigen for the reasons set forth above in item 7. In fact, the evidence presented in Bigotti *et al.*, in particular the fact that these cells do not generally react with anti-class II antibodies, suggests that these cells are not in actuality Langerhans cells, let alone functional antigen-presenting Langerhans cells.

9. It is my understanding that the Examiner alleges that although Bigotti *et al.* do not directly teach that Langerhans cells, in the vicinity of prostate cancer cells capture prostate cancer antigen, and present prostate cancer antigen to T cells, Bigotti *et al.* teaches: i) the presence of presumed Langerhans cells and HLA class II molecules correlates with low grade prostate cancers, as compared with high grade prostate cancers, and ii) such correlation is understandable in view of (a) Langerhans cells and HLA class II molecules can elicit an immune response, capable of direct antigen presentation to immune cells, (b) Langerhans cells act as

antigen presenting cells in neoplastic environment, and (c) HLA class II molecules expressed by neoplastic glandular epithelium, with the aid of Langerhans cells, interact with macrophages and with T helper lymphocytes, and cause expansion of cytotoxic T cells and enhancement of antibody response to membrane-bound tumor associated antigen. As above, Bigotti *et al.* does not teach any more than that the presence of S-100 staining cells can be correlated with low grade prostate tumor grading. The immune mechanism outlined in the conclusion of Bigotti *et al.* is merely speculative and is not a mechanism recognized by cancer immunologists at the time the present application was filed. The mechanism recognized in the cancer field is set forth above in item 7.

10. It is my understanding that the Examiner believes that the suggestion by Bigotti *et al.* that Langerhans cells, being antigen presenting cells and eliciting the immune response, could contribute to controlling cancer growth in prostate cancer clearly provides motivation for making dendritic cells specific for prostate cancer antigen *in vitro*, using the method of making dendritic cells taught by Sallusto *et al.* with prostate antigen, for use in inducing an anti-tumor immune response for treating cancer. In my opinion, the teachings of Bigotti *et al.* provide no motivation to use prostate antigen, including prostate tumor lysate, or a prostate associated antigen, and the like, in the method of Sallusto *et al.* In particular, Bigotti *et al.* is directed to an *in vivo* histology observation and does not provide more than speculation that Langerhans cells found *in vivo* might be involved in some immune response mechanism. There is no evidence in Bigotti *et al.* regarding any antigen that might be picked up and presented by the Langerhans cells, much less that the antigen might be used in an *in vitro* method. At the time of filing the present invention there was no evidence that self-antigens presented by an antigen presenting cell could be administered to patient without inducing an autoimmune response. As such, there is no guidance provided by Bigotti *et al.* that would lead to a combination with the methods of Sallusto *et al.*

11. Further, it is my understanding that the Examiner has dismissed Applicants argument that Bigotti *et al.* did not detect infiltrating lymphocytes which might indicate that antigen presenting cells might have successfully taken up and processed prostate antigen for presentation to naïve T cells. It is also my understanding that the Examiner has asserted that

Applicants have not provided objective evidence or references showing that activated CD4+ T cells and CD8+ T cells and activated B cells producing antibodies have to be present right at the site of the prostate glands. In addition, it is my understanding that the Examiner has alleged that one would have expected that the dendritic cells prepared *in vitro* as taught by the combined art would successfully present the prostate cancer antigen and activate T cells, in view of the teaching of Sallusto *et al.* that culture dendritic cells are most efficient for presentation of antigens, and cause proliferation of T cells, and further in view that it is the properties of dendritic cells to activate CD4+ T cells and CD8+ T cells, as taught by Inaba *et al.* In my opinion there is no reason that the skilled artisan would conclude that evidence or references showing that the location of activated CD4+ cells and CD8+ T cells and activated B cell producing antibodies is relevant to the mechanism of antigen presentation by Langerhans cells in prostate cancer suggested by Bigotti *et al.* or relevant to the mechanism of prostate antigen processing suggested by the Examiner. The Examiner has suggested a mechanism of prostate antigen uptake and processing that is not supported by the evidence provided by Bigotti *et al.* Most importantly, there is no speculation as to this mechanism, as it involves prostate tumor antigens, in Bigotti *et al.* As above, the skilled artisan at the time the present application was filed knew that antigen presenting cells, including Langerhans cells were present in normal glandular tissue, such as prostate tissue. It was also known to the skilled artisan that the antigen presenting cells were present in the tissue to contact and uptake foreign antigens which would activate the antigen presenting cells. Further, it was known to the skilled artisan that the activated antigen presenting cell migrated to, for example, a lymph node where contact with and activation of T lymphocytes, B cells and macrophage took place. It was also well known that it was the T lymphocytes, B cells and macrophage that were responsible for any immune response to the foreign antigen. These is nothing in Bigotti *et al.*, other than their proposed immune mechanism involving macrophage, Langerhans cells and B lymphocytes that would suggest that the Langerhans cells were taking up "prostate tumor" antigens. In my opinion there is insufficient evidence in Bigotti *et al.* to suggest a combination with the methods of Sallusto *et al.* to obtain the presently claimed compositions. Evidence of the presence of CD4+ T cells, CD8+ T cells or activated B cells secreting tumor

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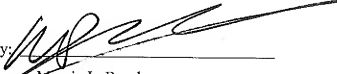
specific antibodies in any part of the body would not add evidence to make the combination of references suggested by the Examiner.

12. Still further, it is my understanding that the Examiner has requested that Applicants provide objective evidence that Langerhans cells are activated and present normal prostate antigen in normal prostate environment. In my opinion, the presence of "activated" Langerhans cells in normal prostate is not relevant to the present compositions or to any prior argument presented by Applicants. The question raised by Applicants is whether Langerhans cells are present in normal prostate and how that number relates to the number of S-100-staining cells found by Bigotti *et al.* This is of some relevance to the rejection made by the Examiner because if there is no change in the number of Langerhans cells between normal prostate and low grade prostate tumor than the various suggestions made by Bigotti *et al.* and the Examiner come into questions. In particular, because Bigotti *et al.* make no determination as to the state of activation of the Langerhans cells. In addition, the Examiner has alleged that Bigotti *et al.* teach that Langerhans cells act as antigen presenting cells in a neoplastic environment. Bigotti *et al.* do not teach that Langerhans cells act as antigen presenting cells in a neoplastic environment, but only offer some speculation as to what the Langerhans cell might do in the environment of a low grade prostate tumor. There is no objective evidence in Bigotti *et al.* that substantiates or even suggests that the Langerhans cells have any function in prostate tissue, much less that the cells have taken in and are presenting prostate antigen to any other cell.

13. It is my opinion that Bigotti *et al.* provides no teachings or suggestions that any prostate antigen has been taken up by the S-100-stained cells found in low grade prostate tumor. As such, there is no reason that the skilled artisan would combine the teachings of Bigotti *et al.* with Sallusto *et al.* in the manner suggested by the Examiner. Further, Inaba (*J. Exp. Med.* 166:182-194, 1987) adds nothing to the teachings of Bigotti *et al.* and/or Sallusto *et al.* that would suggest to the skilled artisan to contact human dendritic cells with a soluble prostate antigen *in vitro* to obtain the composition as presently claimed in the '737 application.

14. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that I make these statements with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of any patent issuing from U.S. Patent Application Number 09/016,737.

Date: 01 Mar 2011

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